Grazing Impacts of Diverse Zooplankton Taxa on Thin Layers

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LONG-TERM GOALS

The US Navy needs to know how distributions and abundances of light-scattering and sound-scattering organisms in the ocean vary in space and time, particularly in the vertical dimension. Recent field observations have shown that many biological properties may vary substantially over small (e.g. centimeter) scales, commonly referred to as "thin layers" (e.g. Cowles et al. 1998, 1999, Hanson & Donaghay 1998, Holliday et al. 1999, Dekshenieks et al. 2001, Alldredge et al. 2002, Rines et al. 2002). Our previous ONR-funded research has allowed us to begin to understand how zooplankton interact with thin layers and how they can take advantage of biomass of prey concentrated in these small-scale features (Avent et al. 1998, Bollens 2000, Bochdansky & Bollens 2004, Clay et al. 2004, Ignoffo et al., 2005). However, there is almost no information regarding how zooplankton can influence the characteristics and persistence of thin layers.

In this project we proposed to address this issue, with two main long-term goals: First, to determine to what extent zooplankton graze and export carbon from thin layers; and second, to determine whether and how zooplankton influence the physical (e.g. optical and acoustical), chemical, and biological characteristics of thin layers with their presence. These goals require determination of rate processes such as feeding activity and excretion, which are very difficult to assess in the field. Thus our research is focused on detailed experimental studies of biological rate processes and behaviors that contribute to the recycling and expert of material in and around thin layers.

OBJECTIVES

The four primary objectives of our proposed research are:

1) To understand the spatial (vertical) coherence and temporal persistence of phytoplankton thin layers with and without the impact of zooplankton grazing.

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2) To understand biological rate processes that influence the carbon budget within and in the immediate vicinity of thin layers.

3) To separate local (within thin layers) from non-local (elsewhere in the water column) effects on the thin layers, depending on the type of zooplankton grazers that utilize thin layer organisms as food sources.

4) To understand to what extent zooplankton return inorganic nutrients to the autotrophs in the layers and thereby influence the persistence and spatial expanse of thin layers.

Our primary hypothesis is that trophic processes alter the flux characteristics of organic carbon and inorganic nutrients such as ammonia and phosphorus in the thin layers and in the immediate vicinity of the layers, thereby changing essential properties of the layers (such as persistence, vertical expanse, biological productivity and export flux). Organisms that aggregate and subsequently stay confined within the layers (e.g. microzooplankton such as ciliates, dinoflagellates or rotifers) will have a different effect on recycling and export fluxes than organisms that only transiently visit these layers (e.g. mesozooplankton such as copepods).

APPROACH

Experimental plankton towers: All experiments are being conducted using a plankton tower tank system installed in the Bollens laboratory at Washington State University Vancouver (Fig. 1). This tower tank system has been used successfully in several previous studies (Speekman et al. 2000, Lougee et al. 2002, Clay et al. 2004, Bochdansky & Bollens 2004, Ignoffo et al., 2005), and was slightly modified for the current project by the addition of valves to allow for 5 cm-spaced subsampling of the tanks in and around the thin layer, installing ethanolamine CO_2 traps to prevent $^{14}CO_2$ release into the atmosphere, and additional high-pressure sodium vapor light sources to increase the range of light intensity for work with autotrophic organisms. In addition to the PI (Bollens) and Co-PI's (Rollwagen-Bollens and Bochdansky), three research technicians from Washington State University Vancouver (Rian Hooff, Angela Gibson, and Celia Ross) have been responsible for the set-up and maintenance of the tower tank system. Mr. Hooff oversees the operation of the tanks and video recording, and Ms. Gibson and Ms. Ross are responsible for culturing all experimental organisms as well as monitoring radiation safety, in consultation with the Washington State University Radiation Safety Office (note: as of July 1, 2007 Ms. Ross has taken over full responsibility for the culturing operations).

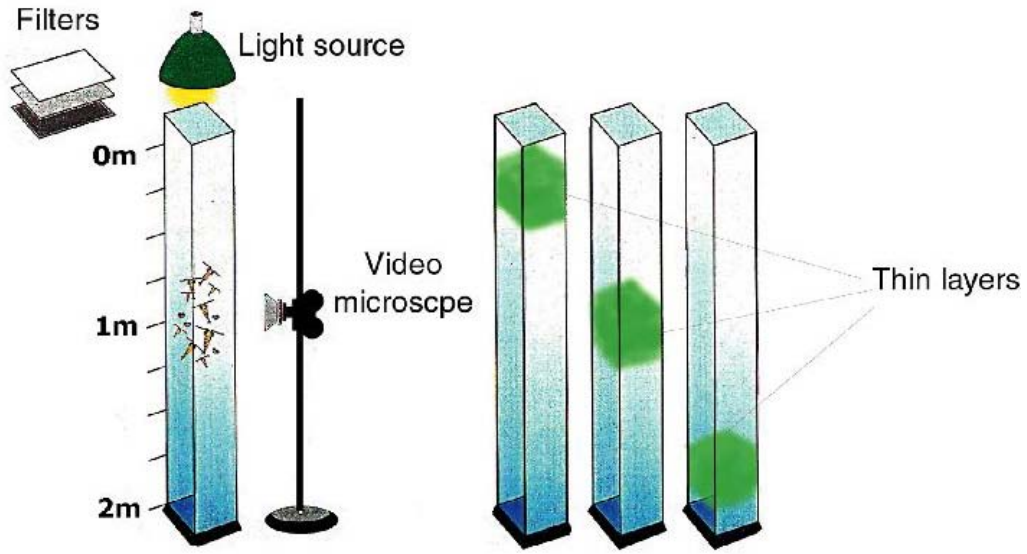

Figure 1. Two-meter high columnar tanks are illuminated by natural light simulators, which incorporate neutral density filters to adjust light intensity. The entire vertical extent of each tank, with a thin layer of phytoplankton, is repeatedly scanned and imaged with an infrared-sensitive video camera to record zooplankton distribution.

Most of the experiments in this research project will be conducted in the first two grant years of the award period (2006 – 2009), which will overlap with portions of the ONR field program: Layered Organization in the Coastal Ocean (LOCO) being conducted in Monterey Bay, CA. The third grant year is currently planned for data analyses, synthesis and publication. Two graduate students (Mr. Joel Quennette from Washington State University and one to be named from Old Dominion University) will work in tandem on the behavioral and physiological aspects, respectively, of the research. In addition, a third graduate student (Ms. Joanne Breckinridge of Washington State University Vancouver) will work on a separate, but related, project examining larval decapod distribution and vertical migration in relation to physical structure (i.e. thin layers) in the water column of estuaries.

Effect of microscale distribution of zooplankton: We are determining the fine-scale distribution of autotrophic and heterotrophic protists in the tanks using an external DFL flourometer (as used in Bochdansky & Bollens 2004), and via direct counts of cells from water obtained through the sampling valves. Mesozooplankton distributions are determined via videomicroscopes that regularly pan the length of the tower tank and record onto VHS tapes. Details on the statistical analyses of distributional data resulting from these experiments can be found in our previous thin layer papers (see references above), as well as in Solow et al. (2000) and Beet et al. (2003). In Year 1 (2005-2006) these "behavioral" experiments were conducted simultaneously with radioisotope experiments to measure the redistribution of carbon in and around thin layers (please see Bollens et al. annual report, September 2006). In Year 2 (2006-2007) the behavioral experiments are being conducted with another set of radioisotope experiments described below.

Redistribution of phosphorus by thin layer organisms (i.e. vertically-migrating phytoplankton): In our Year 1 experiments, we labeled non-migrating phytoplankton cells with ^{14}C and observed the redistribution of labeled carbon through the water column in the presence of micro- vs.

mesozooplankton grazers. In Year 2, we are establishing a nutricline near the bottom of the 2-m tanks using radiolabeled phosphorus (added as $NaH_2{}^{33}PO_4$) in f/2 medium and introducing cultured *Akashiwo sanguinea* (=*Gymnodium sanguineum*) to the surface layer containing nutrient-free seawater. As in previous experiments, we also introduce a salinity gradient to the center of the tanks in order to avoid physical mixing of the phytoplankton with the surrounding water (Bochdansky & Bollens 2004). *Akashiwo sanguinea* is a highly motile mixotrophic dinoflagellate that commonly forms dense surface and subsurface aggregations in Monterey Bay and other estuaries on the US west coast (Horner et al. 2001). Notably, during the 2005 LOCO field program, *A. sanguinea* was the dominant thin-layer organism. *Akashiwo sanguinea* has also been observed to vertically migrate in the water column from the surface into the nutricline in coastal bays and estuaries, including Monterey Bay Donaghay et al. 2007).

Phosphorus taken up by *A. sanguinea* in the lower part of the tanks may be incorporated into biomass and/or excreted or defecated elsewhere in the water column. In addition, phosphorus may move upward in the tanks due to passive diffusion. The redistribution of phosphorus is monitored over time (48 hours) at 6-12 hour intervals and over the space of the 2 m tower tanks by taking samples through valves positioned along tanks' walls. The samples are filtered over GF/F filters to separate the dissolved and particulate fractions, then further subsampled and processed to distinguish inorganic phosphorus from total phosphorus, with organic phosphorus determined by difference. These steps are followed to track radiolabelled phosphorus (33-P) as well as non-labelled phosphorous (Fig. 2). Isotope analysis is being conducted using a Packard Tri-Carb 2100 TR liquid scintillation counter. Soluble reactive phosphorus was determined using the ammonium molybdate reagent (Parsons et al. 1985). Total dissolved phosphorus (including organic phosphorus) and particulate phosphorus on GF/F filters were determined after muffling and acid digestion (Solorzano & Sharp 1980) All instruments are housed at Washington State University Vancouver.

WORK COMPLETED

We performed four sets of experiments in June 2007. The first experiment was run "cold" (i.e. without radiolabelled phosphorous) in order to assess *Akashiwo sanguinea* behavior and determine the appropriate nutrient levels, etc. The remaining three experiments were all conducted with radiolabelled 33-P. Each experiment consisted of a control tank (nutricline with no *Akashiwo sanguinea)* and three treatment tanks, each with a nutricline and identical concentrations of *A. sanguinea* but varying light levels (low, medium, and high). Nutrient concentrations in the lower part of the tank were set to mimic values observed in Monterey Bay during LOCO cruises in 2005 (data courtesy Al Hanson, SubChem Systems).

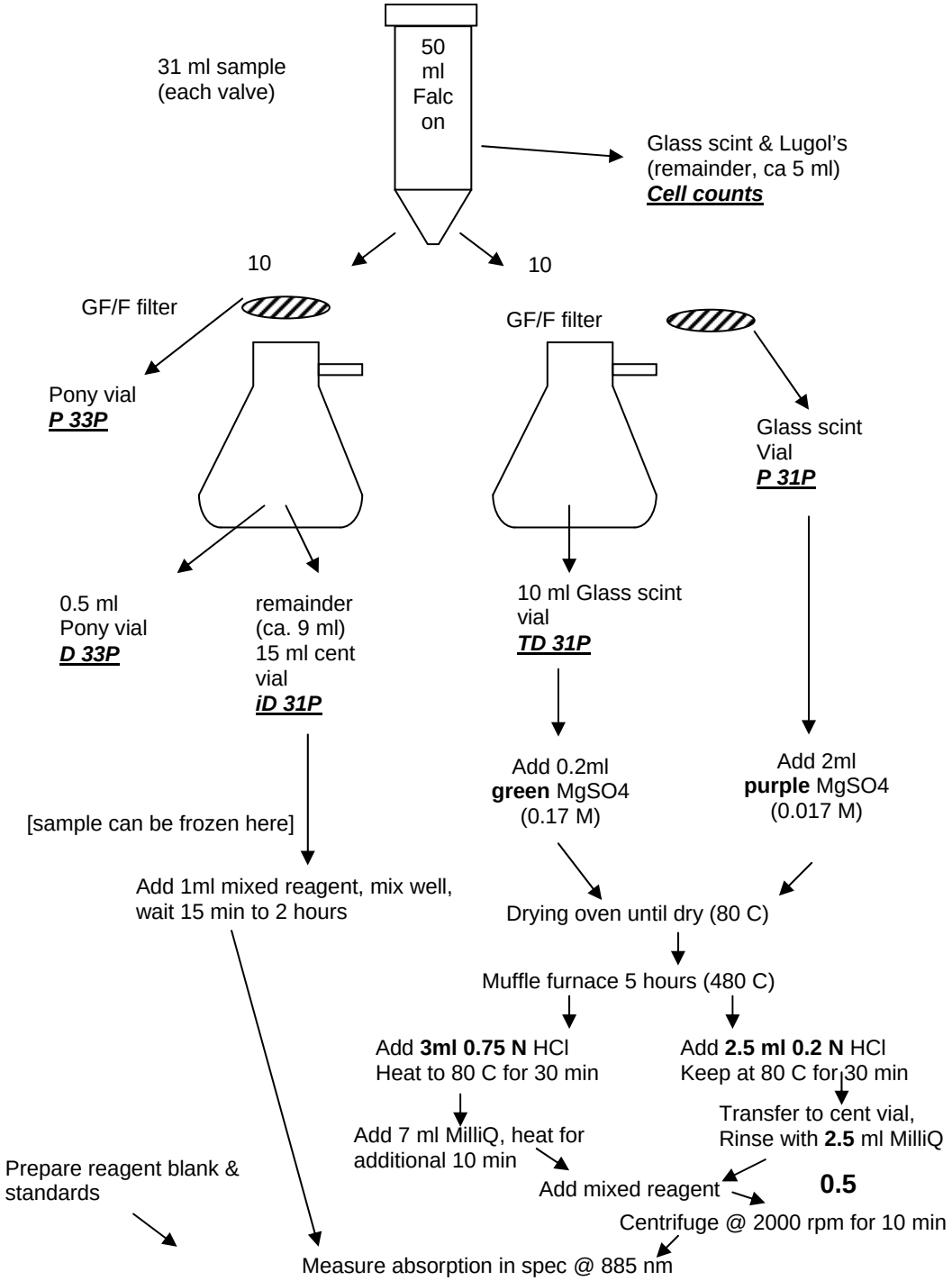

Figure 2. Flow chart illustrating the sample processing procedures to fractionate dissolved and particulate phosphorous, and further measure inorganic dissolved/ particulate phosphorous and total dissolved/particulate phosphorous concentrations.

RESULTS

We observed a difference in the day and night vertical distributions of *Akashiwo sanguinea* in the low and medium light treatments as compared to the high light treatment (Fig. 3). *A. sanguinea* in the low and medium light tanks showed a clear migratory pattern, aggregating near the surface during the day (noon) and distributed evenly through the lower part of the 2-m water column at night (midnight). In addition, the migration appeared to be anticipatory, i.e. upward migration began before the lights came on or off. Conversely, *A. sanguinea* in the high light treatment were distributed more evenly through the tank and with a smaller peak at the nutricline during both day and night. This suggests that the dinoflagellates were either light inhibited and not migrating, or that they had sufficient light at all depths and thus migration was not necessary. Discerning between these, or other possible, explanations will be the focus of additional behavioral experiments in Year 3.

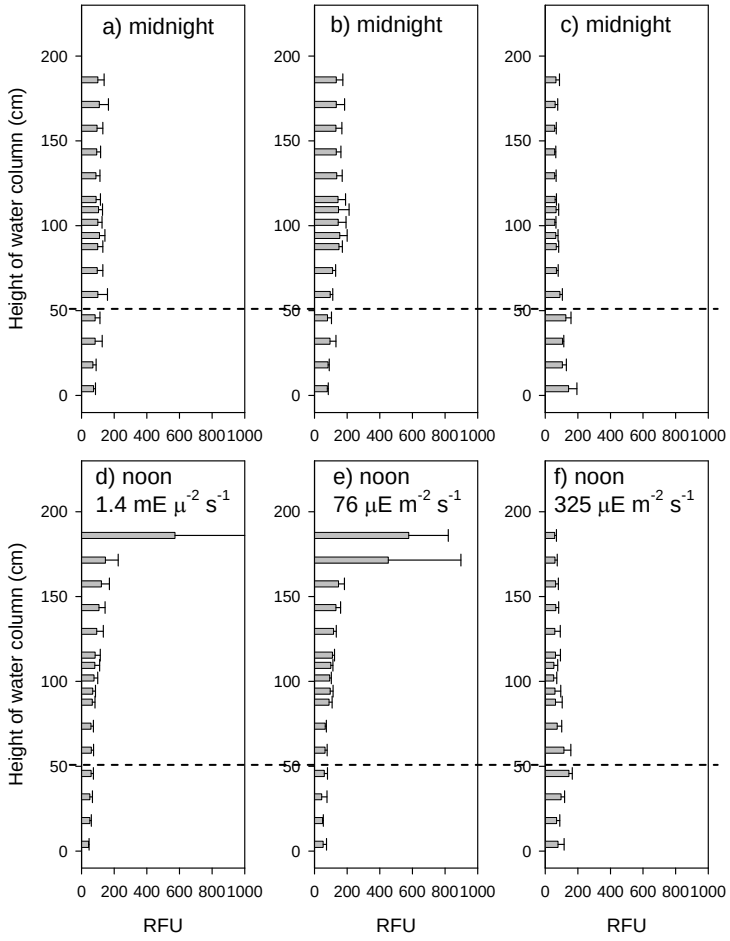

Figure 3. Vertical migration of Akashiwo sanguinea in experimental tanks. Distibution of A. sanguinea is shown as relative fluorescence units (RFU) using a fluorometer through the tank walls, tracking the distribution of the dinoflagellates without disturbing organisms or nutrient gradients. A. sanguinea forms dense layers at the surface during the day when light is limited (d & e). When light is sufficiently strong at the nutricline (dashed line), A. sanguinea stays at depth during the day (f). Figures on top of each other depict the same tank.

We also observed a distinct difference in the net uptake of dissolved 33-P by *Akashiwo sanguinea* depending on light level relative to the control (Fig. 4). In all three treatments (low, medium and high light) there were very small increases in dissolved 33-P in the upper water column. However there were substantial net losses of dissolved 33-P in the lower water column in all treatments, indicating utilization of 33-P in all tanks with *A. sanguinea* present, with the degree of net loss increasing with increasing light level.

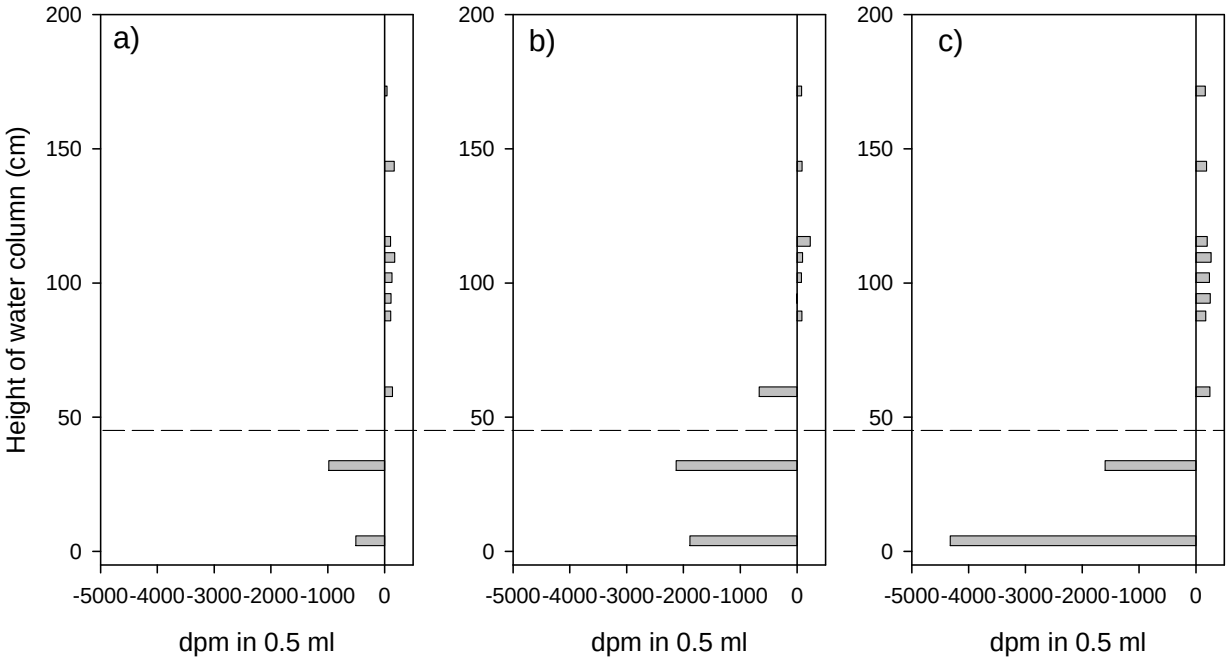

Figure 4. Net changes in 33-P content in the dissolved fraction in comparison to a control tank not containing Akashiwo sanguinea. Phosphorus is taken up at depth (below the nutricline; dashed line) and moved towards the top of the water column in an upwards biological pump due to the migration of A. sanguinea. The released dissolved organic material is composed of both inorganic and organic phosphorus. The strength of the phosphorus transfer is light dependent: a) 1.4 $\mu E\ m^{-1}\ s^{-1}$, b) 76 $\mu E\ m^{-2}\ s^{-1}$, c) 325 $\mu E\ m^{-2}\ s^{-1}$.

Finally, we calculated the net incorporation of phosphorus into *Akashiwo sanguinea* (particulate fraction) over 48 hours for each experiment (correcting for isotope "dilution" with the non-labelled 31-P). These results further demonstrate the differing effect of light level, with higher incorporation rates in the high light treatment compared to the low and medium light treatments (Fig. 5).

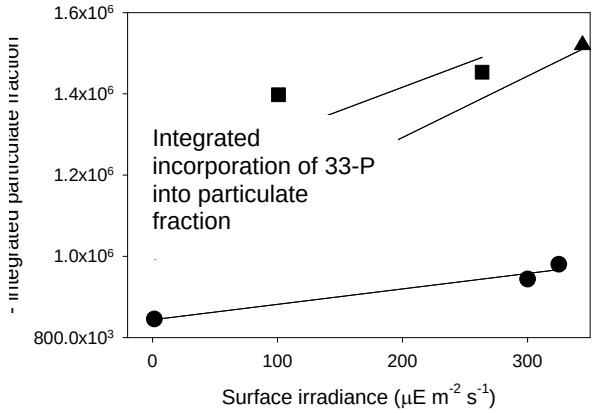

Figure 5. Isotope tracer experiments with the migrating dinoflagellate Akashiwo sanguinea. Water column-integrated incorporation of 33-P into the particulate fraction is dependent on irradiance levels over a 48 hour time course. Circles: experiment 1, triangles: experiment 2, squares: experiment 3. For comparisons, experiments were corrected for radioactive decay of 33-P (half life of 25.4 days), and for the molar isotope dilution of 33-P in the 31-P medium. Experiments differed in cell concentrations of A. sanguinea which is reflected in the elevations of the regression lines.

IMPACT/APPLICATIONS

This research will be an important contribution to the Thin Layers program, as it directly addresses the influence of zooplankton on rate processes in thin layers, and moreover addresses the influence of migrating thin layer phytoplankton on the distribution of major nutrients within and around thin layers. While field studies have to rely primarily on inference from distributions, our controlled laboratory experiments will provide flux patterns of important inorganic and organic nutrients in and around thin layers. In this experimental setting we are able to manipulate predator – prey ratios and available nutrients. We will therefore be able to understand potential effects of zooplankton on the persistence and internal dynamics of thin layers, as well as the effect of thin layer phytoplankton themselves. Our experimental work will provide sufficient data to allow us to make predictions about the contribution of biological processes to thin layer dynamics given the presence and abundance of various plankton species found in the field.

RELATED PROJECTS

This research is relevant to virtually all of the many field studies previously and currently being undertaken within the “Thin Layers” program.

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